SEROLOGICAL IDENTIFICATION OF PORCINE MYCOPLASMAS

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Growth inhibition and epi-immunofluorescence of Mycoplasma colonies have proved to be simple and specific tests for classification of various mycoplasmas species. However, as it requires high-titered serum, the disc-growth inhibition test is often insensitive and in spite of the fact that immunofluorescence is a very satisfying method, the identification of *Mycoplasma hyopneumoniae* colonies by fluorescent antibody staining is sometimes difficult to realize because the colonies wash off the surface of the agar.

Whereas the active antibodies in growth inhibition test are induced by membrane components (KAHANE and RAZIN, 1969 ; HOLLINGDALE and LEMCKE, 1969 ; LEVISOHN and RAZIN, 1973 ; GOEL, 1973), the objectives of this investigation were to develop practical performance of the growth inhibition test using membrane antigens for identification of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*.

Five strains of *Mycoplasma hyopneumoniae* and five strains of *Mycoplasma hyorhinis* isolated in France and identified by growth inhibition and immunofluorescent tests* were grown as described by FRIIS, 1975. The cells were harvested from the liquid medium during their late logarithmic phase by centrifugation at 20,000 x g for 1 h at 4°C. The sediment was then washed thrice with 0,1 M Tris-hydrochloride buffer (pH 8,0). For membrane preparations, the cells were lysed by sonication and the membranes were recovered by centrifugation at 50,000 x g for 1 h at 4°C. They were then washed three times with 0,1 M Tris-hydrochloride buffer (pH 8,0) and purified (MARCHESI et al., 1970). The membranes were finally washed once with distilled water and lyophilised. Rabbits were immunized by subcutaneous injections with a suspension of cells or a suspension of membranes mixed with Freund adjuvant as described by WROBLEWSKI, 1975. The reproduction of antibodies was checked weekly by cross immunoelectrophoresis experiments after the fourth inoculation.

Six mm diameter discs of filter paper (Disks 1/4, Difco) were moistened with 25 µl of undiluted antiserum, dried and placed on the agar seeded with a suspension of homologous and heterologous organisms (inoculum containing 10^3 cfu/ml). The inhibition zones were measured in mm from the edge of the discs to the rim of the growth after 72 hours.

TABLE I - growth inhibition test :
. I : sera with antibodies against *Mycoplasma hyopneumoniae*
. II : sera with antibodies against membrane preparations

	Mycoplasma hyopneumoniae	ANTISERA				
		Anti 1	Anti 2	Anti 3	Anti 4	Anti 5
I	Strain 1	5	4	5	4	4
	Strain 2	4	4	3	4	3
	Strain 3	3	3	4	4	4
	Strain 4	4	3	4	4	4
	Strain 5	3	2	3	2	3
II	Strain 1	9	8	9	8	8
	Strain 2	9	10	8	9	8
	Strain 3	8	8	8	7	8
	Strain 4	9	9	8	9	8
	Strain 5	9	8	8	8	9

* *The identification of the 10 strains was confirmed in the Institute of Medical Microbiology, Aarhus, DENMARK.*

TABLE II - Growth inhibition test :
I : sera with antibodies against *Mycoplasma hyorhinis* cells
II : sera with antibodies against membrane preparations

	Mycoplasma hyorhinis	ANTISERA				
		Anti 1	Anti 2	Anti 3	Anti 4	Anti 5
I	Strain 1	3	2	2	2	3
	Strain 2	3	3	3	3	2
	Strain 3	2	3	3	3	2
	Strain 4	3	2	3	3	2
	Strain 5	2	2	3	2	3
II	Strain 1	8	6	8	6	6
	Strain 2	5	7	5	7	6
	Strain 3	7	6	7	6	6
	Strain 4	6	6	6	7	7
	Strain 5	6	6	6	6	7

As seen in table I and Table II, antisera containing antibodies induced by membrane components (antisera II) increased the size of inhibition zones. In *Mycoplasma hyopneumoniae* species, homologous reactions shown results ranged from 8 mm to 10 mm and from 7 mm to 8 mm in *Mycoplasma hyorhinis* species. When antibodies against mycoplasma cells (antisera I) were used, inhibition zones were 3 mm (*Mycoplasma hyorhinis*) and from 3 mm to 5 mm (*Mycoplasma hyopneumoniae*). No cross reaction occured between *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*. In the same species, no significant difference was obtained between homologous reactions (antiserum 1 against strain 1) and heterologous reactions (antiserum 1 against strain 2).

CONCLUSION : On the basis of the present investigations it can be concluded that growth inhibition test using antibodies induced with membrane antigens is well suited for serological identification of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*.

SELECTED REFERENCES : FRIIS, N. F. (1975), Nord. Vet. Med., 27, 337-339 ; GOEL, M. C. (1973), J. Bacteriol, 116, 994-1000 ; HOLLINGDALE, M. R., and LEMCKE, R. M. (1969), J. Hyg. 67, 585-602 ; KAHANE, I., and RAZIN (1969), J. Bacteriol, 100, 187-194 ; LEVISOHN, S. and RAZIN, S. (1973), J. Hyg., 71, 725-737 ; MARCHESI, S. L., STEERS, E., MARCHESI, V. T. and TILLACK, T. W. (1970, Biochemistry, 9, 50-57 ; WROBLEWSKI, H. (1975), Biochimie, 57, 1095-1098.